

Title	Stemness ontology and therapeutic strategies (Proceedings of the CAPE International Workshops, 2012. Part I: IHPST, Paris - CAPE, Kyoto philosophy of biology workshop)
Author(s)	Laplane, Lucie
Citation	CAPE Studies in Applied Philosophy and Ethics Series (2013), 1: 77-99
Issue Date	2013-02-12
URL	<a href="http://dx.doi.org/10.17983/203246">http://dx.doi.org/10.17983/203246</a>
Right	
Type	Departmental Bulletin Paper
Textversion	publisher

# Stemness ontology and therapeutic strategies

## Lucie Laplane

Cancer stem cells are the new targets of the “war on cancer” (Lenz 2008; for the “war on cancer” see the US National Cancer Act signed by President Richard Nixon). After 40 years and the 105 billion dollars spent by the National Cancer Institute alone, improvement has been low (less than 5%), cancers are still there, killing thousands of people every days, while other important causes of death like stroke and cardiovascular diseases have substantially declined (improving by more than 64%) (numbers from Kolata 2009; see also the well-known *Facts & Figures* of the American Cancer Society). Despite undeniable progress, the comparative failure of oncology has put the whole therapeutic classical enterprise under suspicion. This context clearly opened a window for proposed original therapeutic strategies that would give hope of “breaking the cancer war stalemate” (Haber et al. 2011, p.19). Thus, when the role and existence of the so called cancer stem cells have been brought on the table with the proposition of an entirely new and very heuristic therapeutic strategy against cancers, it didn’t take long to spread. Cancer stem cells (CSCs) are cancerous cells with stemness properties. According to the cancer stem cell model of carcinogenesis, which is based on accumulating evidence, the CSCs are the only tumorigenic cells of the cancers. This means that cancers are initiated, developed and spread by the CSCs and never by any other cancerous cells. From this model of carcinogenesis, biologists have deduced (1) that relapses after apparently successful therapies are due to resistance of few CSCs and (2) that it would be necessary and sufficient to kill those CSC in order to cure cancer.

In this chapter, we are going to question this therapeutic strategy and show that it relies on historico-ontological choices, which remain to be supported. For this purpose we are first going to depict the coming into being of the cancer stem cell theory. We are then going to highlight the ontological perspective that has been raised by the CSC biographies. Other stem cells biographies have raised other competing ontological

perspectives on stemness. This state of competition will lead us to analyze the consequences of those distinct ontologies of stemness on the CSC targeting strategy.

## **Cancer stem cells biographies**

Cancer biology is highly complex. It gathers together heterogeneous diseases affecting various parts of the organism at diverse levels of organization (genetic and epigenetic, molecular and cellular) and involving a multitude of genes and molecular pathways. This complexity is argued to explain why a cure is still lacking: brain cancers are very distinct from blood cancers, among which acute myeloid leukemia are different from chronic lymphoid leukemia, which themselves can result from various deregulations of normal functions. There is thus very little hope to find a universal cure.

The classical therapeutics, surgery apart, involve anti-mitotic agents. They destroy cells in proliferation (which result in various side effects like hair loss, digestive disorders and blood aplasia). This therapeutic strategy came from the common apparent problem of proliferation involved in every cancer. Indeed, cancers cells are assumed to escape the steady state of the organism and outgrow. Thus, cancer cells have been thought to divide a lot. But this has been proven wrong since the 50's: cell division is equivalent in cancers and in steady-state tissues. A lot of cancer cells are not dividing (Astaldi et al. 1947; Astaldi and Mauri 1950; Killmann et al 1963; Mauer & Fisher 1966). This observation has been explained by two concurrent hypotheses: either all cancer cells have the ability to divide but do so at different stochastic rates, or most cancer cells do not divide but there is a pool of stem cells that feed the cancer cells population. Those two explanations have been called respectively “self-maintaining system hypothesis” and “stem cells hypothesis” (Clarkson 1969; Clarkson et al. 1970; Gavosto et al 1967a and 1967b). For leukemias, the latter hypothesis has gained support from clonogenic studies. Those deserve historical emphasis.

## **Leukemic and hematopoietic stem cells**

The nuclear area led to fear of major radiations (which could be caused by nuclear

wars, by fallout from nuclear weapon testing or by production accidents –all these cases had occurred). The first cause of death after non-directly lethal radiations was blood depletion. Thus biologists began to actively search a way to rescue the hematopoietic system by transplantations of bone marrow cells (Lorenz et al. 1951; Kraft 2009). These led to the constitution of *in vitro* and *in vivo* assays which allow studying the capacity of a single cell to produce an entire population of cells, called a “clone” (Till & McCulloch 1961; Becker et al. 1963). Using these assays, biologists have highlighted the existence of a hierarchy of cells with distinct clonogenic abilities in both normal blood system and leukemias (Till & McCulloch 1980; Metcalf et al. 1969; McCulloch 1983; Griffin & Löwenberg 1986). At the top of the hierarchy are the so-called “hematopoietic stem cells” and “leukemic stem cells” respectively. These cells are highly able to produce clones containing different kind of cells. But it rapidly appeared that the most immature cells detectable by these assays weren’t the true hematopoietic and leukemic stem cells (Dick 2008; Griffin & Löwenberg 1986; Buick et al. 1979; McCulloch & Till 1981).

In the 90’s, the new hopes raised by gene therapies to cure blood genetic diseases caused by single-gene defects urged the identification of hematopoietic stem cells. Indeed, the aim of gene therapies is the “permanent correction of genetic deficiencies of the hematopoietic system” (Larochelle et al. 1995, p. 163). This necessitates transducing the right target, which is the hematopoietic stem cell. Otherwise the disease is likely to reappear (Larochelle 1996). The development of three technologies played a major role in this race to the identification of hematopoietic stem cells: production of strains of immunodeficient mice in which xenotransplantation of human cells was possible, FACS technologies (Fluorescence-Activated Cell Sorting), and production of monoclonal antibodies. The latter two, once combined, allow cell sorting according to their cell surface antigens. Immunodeficient mice were highly needed for modeling and studying blood cancers, HIV, and immune disorders (see Schults et al. 2007; Belizário 2009). A lot of strains were rapidly produced among which the NOD-SCID mice (Non Obese Diabetic- Severe Combined Immunodeficiency) played a major role in the identification of human hematopoietic and leukemic stem cells (Dick 1996).

The coming into being of the FACS technology was also very exciting. This ingenious technology was developed with Becton Dickinson Electronics’ collaboration

on the model of ink jet printing technology, which is able to generate droplets and change their direction. Its development has benefited from two impetuses of automation. The first one was the need of automation to standardize clinical-diagnostic practice. The second one came from the NASA research in exobiology (Cambrosio & Keating 1992a; Keating & Cambrosio 1994). In combination with the production of monoclonal antibodies from the hybridoma technology developed by immunologists (Cambrosio & Keating 1992b), FACS can sort hematopoietic and leukemic cells according to their antigens. Immunologists and hematologists used it for the research of the hematopoietic stem cell (see Fagan 2007 for the history of identification and characterization of the cells of the hematopoietic hierarchy, including stem cells, by Irving Weissman research group). They sorted the cells, transplanted them into lethally irradiated immunodeficient mice, and then studied their ability to rescue the hematopoietic system. From these studies emerged the first phenotypic characterization of the human hematopoietic stem cells: these are CD34+CD38- (Larochelle et al. 1996; see also Weissman et al. 1989). Identification of the leukemic stem cells followed few months later with the same phenotypic characteristics (Bonnet & Dick 1997). This protocol (cell sorting and transplantation in immunodeficient mice) rapidly became a gold standard for the isolation and characterization of any kind of “adult” or “somatic” stem cells.

### **Origin of solid cancers in immature cells: The embryonic rests theory**

The very idea of the existence of cancer cells that would be stem cells, and that would be at the origin of cancers, wasn't restricted to blood cancers. Indeed, since the cell theory from the XIX<sup>th</sup> century, there have been some proponents of various interpretations of embryonic/undifferentiated/stem cells in cancers that are historically linked and consistent (even if different in many aspects) with the modern current notion of cancer stem cells. Rudolph Virchow, who formulated the second principle of the cell theory “*omnis cellula e cellula*” (the first one being that organism are made of cells; see Schwann 1839; Schleiden 1838; Remak 1855; for an historical perspective see Duchesneau 1987) also claimed that tumors are developed from the transformation of

normal cells (Virchow 1858). But some cases of cancers then appeared struggling to explain, like epithelial cancers located in bones. According to the germ layer theory, each cell of the adult organism comes from one of the three layers of the embryo (endoderm, mesoderm and ectoderm) and no crossing is possible in the fate of the cells coming from each layer (Remak 1855). This, in consequence, made impossible the development of epithelial cells and epithelial canceroids from bone cells since those two kinds of cells come from distinct germ layers (Hannover 1852, see in particular p. 51 and 21; Lebert 1851). Interestingly, two explanations of occurrences of epithelial canceroids in bones were suggested which both involved undifferentiated cells. They are often erroneously indistinctly seen as “the embryonal rest theory”.

The very embryonal rest theory has been suggested by Robert Remak, and holds that tumors of that kind (i.e. epithelial canceroids located in tibias, or any other bone of the appendix skeleton for that matter) would be derived from embryonic cells of their own layer, which would have been delocalized during the early development of the embryo (Remak 1854, see in particular p. 172). This hypothesis has later been generalized to every cancer by Julius Cohnheim (Cohnheim 1875 and 1889, see p. 760 and forward) and disseminated (see for examples, Durante 1874; Askanazy 1907; Budde 1926; Willis 1948).

The other explanation is quite different. It was developed by Rudolph Virchow and his colleague August Fœrster, who didn't put so much emphasis on the germ layer theory. They were among those pathologists who had questioned the pertinence of the embryological dogma under pathological contexts (see also Klebs 1867; Langhans 1867). They both argued in favor of an origin of most cancers from cells of the connective tissue, which they saw as a “germinal source” (Virchow 1855: 415), a bank of undifferentiated cells that provide resources for the emergence of neoplasms and neoformations (Virchow 1858, see p. 355; Rather 1978, see p. 131; Fœrster 1855, see pp. 180-181 and p. 208).

The theory of the origin of cancers in undifferentiated cells of the connective tissue rapidly fell in desuetude (for an analysis see Rather 1978, p. 154). But the embryonic rest theory retained attention during the XX<sup>th</sup> century in particular for the explanation of adamantinomas of the appendix skeleton (the epithelial cancer that had been at the

origin of the proposition of the rest theory) and of teratomas and teratocarcinomas. Adamantinoma is still a matter of controversy today. The theory of embryonic rests, and some modified version of it have been repeatedly suggested to explain their origin (Fischer 1913; Richter 1930; Ewing 1940; Schulenburg 1951; Brunner 1936; Lauche 1947; Rosai 1969; Spjut et al. 1971; Jain et al. 2008). The teratomas and teratocarcinomas are tumors that are characterized by the presence of very heterogeneous kinds of cells derived from the three germ layers. The historian Andreas-Holger Maehle reported that numerous experiments were achieved in the early XX<sup>th</sup> century in order to test Cohnheim theory, in particular through tentative of transplantation of embryonic cells (for details and references see Maehle 2011). Max Askanazy, notably, obtained teratoids by injecting embryonic cells into the abdominal cavity of rats, which was according to him a “beautiful” illustration of Cohnheim’s theory (Askanazy 1907; Maehle 2011). He was the first to use the concept of “stem cells” (*Stammzellen*) for these residual embryonic cells from which teratocarcinomas would originate. Leroy Stevens and collaborators came with the proof of concept that teratomas could originate from embryonic cells in the late 60’s by transplanting cells of young mice embryos into testis of adult mice and obtaining development of teratomas from the transplanted cells (Stevens 1968, 1970; Dunn & Stevens 1970; for a review see Damjanov & Solter 1974). Interestingly enough, development of teratomas is now considered as a proof of pluripotent embryonic stem cells. In the mean time, Lewis Kleinsmith and Gordon Barry Pierce of the Pathology Department of the University of Michigan were transplanting the teratocarcinoma cells that look like embryonic cells (termed “embryonal carcinoma cells”) to study their potentiality. Pierce and his colleagues first demonstrated, against the popular belief, that teratocarcinomas are produced by differentiation of multipotential cells (Pierce & Dixon 1959; See also Arechaga 1993). He then highlighted the multipotentiality of the cells in which cancers originate. He showed that this multipotentiality was restrained to a tiny subpopulation of cancer cells, and claimed that “neoplasms consist of a heterogeneous population of cells, a small number of which are undifferentiated, highly malignant stem cells; the rest are less malignant and more differentiated”. (Kleinsmith & Pierce 1964: 1548). Pierce himself interpreted his works as a continuity of the embryonal rest theory, often making

references to Cohnheim, Askanazy, Budde and Willis. From these first studies, Pierce built a general theory of carcinogenesis as a caricature of normal development, supported by studies on other cancers, in particular breast and colon cancers (Pierce 1967; Pierce 1974; Pierce 1977; Pierce et al. 1977).

## **The cancer stem cell theory at the confluence of these biographies**

A general cancer stem cell theory finally resulted from these biographies, claiming three distinct and complementary theses (see in particular Reya et al. 2001 and Clarke et al. 2006):

- 1) Cancers are hierarchically organized: they are initiated, developed and propagated exclusively by a specific sub-population of cancer cells, the so-called “cancer stem cells” (CSCs).
- 2) CSCs explain relapses after apparent healing because (a) they can escape and/or resist to therapies, and (b) they are highly able to initiate a relapse.
- 3) Targeting those CSCs is necessary and sufficient to permanently cure cancers.

These theses raised back hopes to find real cures for cancers. These hopes have been taken very seriously, since there has been a profusion and accumulation of data in favor of the existence of CSC in various cancers such as leukemias (Bonnet & Dick 1997; Cozzio et al. 2003), breast cancers (Al-Hajj et al. 2003), brain tumors (Singh et al. 2003; Singh et al. 2004), prostate cancers (Collins et al. 2005; Lawson et al. 2007), ovarian cancers (Szotek et al. 2006), liver cancers (Suetsugu et al. 2006; Chiba et al. 2007), colorectal cancers (Dalerba et al. 2007; O'Brien et al. 2007; Ricci- Vitiani et al. 2007), pancreatic cancers (Li et al. 2007; Hermann et al. 2007), head and neck squamous cell carcinomas (Prince et al. 2007), lung cancers (Eramo et al. 2008)... Now a day, the development of drugs specifically targeting CSCs raises lots of private and public funding. Biotech companies specialized on the R&D of CSC targeting therapeutics are blooming since few years, and investments of big pharmas are considerable. IPOs are going well, showing a real optimism for the CSC targeting therapeutic strategy. All of them seem to shout in unison “off with their head”, convinced that killing CSC might solve the cancer problem. But are they right? Or are they like the “blind fury” queen of



hearts character in Lewis Carroll's *Alice Adventures in Wonderland*?



## Ontology matters

Cancer stem cell became a well-established object around the very beginning of the XXI<sup>st</sup> century. As we have shown, this coming into being followed a century of more or less dispersed inquiries and researches, in which CSCs were often studied hand in hand with hematopoietic stem cells. But they are not the only stem cells, and their biographies are far from subsuming the biographies of others objects also called stem cells, like the embryonic stem cells (ES), the induced pluripotent stem cells (iPS) and the totipotent stem cells. The multiplicity of stem cells, each with their own biographies, resulted in some problems of definitions.

Indeed, stem cells are classically defined in text-books as those cells that have two properties: the capacity to self-renew and the ability to differentiate into two or more cell types. This definition is meeting accumulating problems with the profusion of types of stem cells. Several biologists had criticized it for being either too inclusive or too exclusive. Both criteria appeared too inclusive for the following reasons: (a) some non-stem cells like lymphocytes can also self-renew (Mikkers & Frisen 2005, Zipori 2004; Younes et al 2003), and (b) some non-stem cells like many progenitors (or transit-amplifying cells) can also differentiate into two or more different cell types (Lander

2009). A possible response to this problem of inclusivity is to consider that stem cells possess the two properties whereas (a) progenitors do not self-renew (or not for long anyway), and (b) lymphocytes do not differentiate into different cell types (see for example Seaberg and van der Kooy 2003). But then, the problem is that together these two properties, used to define stem cells, become too exclusive: (1) some stem cells, such as mammal embryonic stem cells, do not self-renew *in vivo* (Lander 2009), indeed they belong to transient “emergent tissues” and are part of the organism for a short period of time (Shostak 2006) and (2) “there are unipotent self-renewing cells, most notably germ-line stem cells, which most scientists would argue are obvious stem cells” (Mikkers and Frisen 2005: 2715). One possibility to resolve this problem is to consider that germinal stem cells, embryonic stem cells, and all the cells that do not share the two properties are not real stem cells. A few scientists follow this path. But it is to be noted that they are never working on embryonic or germinal stem cells. This solution does not lead to a consensus.

The stem cell concept has met another crisis during the past decade. The increasing needs of stem cells for medical purposes, like regenerative medicine or gene therapies, led to a race to the precise phenotypic characterization of the stem cells. It also led several groups of biologists to the search of a “stemness signature”. This search relied on two major well-implanted presuppositions. The first one was that the two defining properties of stem cells (self-renewal and differentiation potency) allow a qualitative distinction between stem cells and non-stem cells. This presupposition has met the critics we highlighted below. The second one was that these two properties might be reducible to molecular characteristics. This presupposition relies on a rather simple idea: “Because all SCs share fundamental biological properties, they may share a core set of molecular regulatory pathways” (Ivanova et al. 2002, p. 601). This led three major groups of the stem cell research community to the search of a genetic characterization of *stemness*. Ihor Lemischka’s group at Princeton compared genetic profile of human and mice hematopoietic stem cells and found 283 shared highly expressed genes. They thought some of these genes are constitutive of the stemness “genetic program” (Ivanova et al. 2002, p. 604). Douglas Melton’s group at Harvard has compared transcription profiles of embryonic, neural and hematopoietic mouse stem cells and found a list of 216

highly expressed genes (Ramalho-Santos et al. 2002). Finally, Bing Lim's team from the Genome Institute of Singapore, compared gene expression profiling of embryonic, neural and retinal stem cells. They found a list of 385 common genes. This last group compared their data with the two previous groups and found only one common gene: integrin- $\alpha$ -6 (ITGA6) (Fortunel et al. 2003). ITGA6 gene codes for the  $\alpha$ 6 subunit of the  $\alpha$ 6 $\beta$ 4 transmembrane protein which is not at all specific to stem cells. Indeed, it is primarily found in epithelial differentiated cells (see the Genetics Home Reference of NIH).

These studies have engendered a debate in the scientific community. Several interpretations of this failure have been suggested. A popular one consists in imputing the failure to the experimental settings (Burns & Zon 2002; Ivanova 2003; Vogel 2003). According to this view, scientists should persist so that the "current impressionistic portrait of a stem cell may then transform into realism" (Burns & Zon 2002, p. 613). The other one, far less popular, consists in considering that "there is no such thing as intrinsic stemness at the molecular level, such that perhaps stemness should be understood as a relational property between cells and their microenvironment generating the functionality of stem cells." (Robert 2004, p. 1007) This approach has been investigated by trying to determine the role of the microenvironment in the fate of stem cells, i.e. in the choice between self-renewal and differentiation (see for example Hackney et al. 2002). The very project of reducing *stemness* to some genetic characteristics also meets another problem. Contrarily to the differentiated cells, the two functional properties that define stem cells are rarely actualized because stem cells divide seldom (they are often quiescent). Thus, supposing that those two "fundamental biological properties [...] share a core set of molecular regulatory pathways" as suggested by Ivanova et al, and supposing that those molecular characteristics were identified, those couldn't be sufficient to define or portray stem cells because such a characterization would miss all the non-dividing stem cells (i.e. a lot of them). This is a worth-asking question.

Last but not least, the well implanted idea that differentiation was a one-way street going from stem cells to differentiated cells, with no coming back, suffered from various challenges, beginning with cloning and increasing lately with iPS cells technologies and transdifferentiation (for an historical perspective, see Maienschein 2002). These

challenges are often minimized by biologists, arguing that they involve tremendous experimental settings. That is to say, these data are restricted to *in vitro* conclusions. They do not challenge the one-way differentiation *in vivo*. But there are now accumulating data challenging this dogma *in vivo* too. There has been some appealing data coming from the regeneration field exhibiting processes of dedifferentiation before regeneration (see for example Lo et al. 1993). But those were restricted to few species. Since then, it has been proven that in drosophila and mice testis and ovary, spermatogonia and cytotocytes do dedifferentiate into germline stem cells under certain circumstances (Brawley & Matunis, 2004; Kai and Spradling, 2004; Barroca et al. 2009). Chaffer et al. (2011) have showed that non-stem epithelial cells of the breast can spontaneously dedifferentiate to a stem state. They have also highlighted that this phenomenon is increased in cancers, where non-stem cancer cells can give rise to cancer stem cells *in vitro* as well as *in vivo*. The observation of the occurrence of epithelial-mesenchymal transitions had been followed by the observation that they might cause a *de novo* acquisition of stemness (Mani et al. 2008). Breast CSCs have been generated by this process (Morel et al. 2008). In fact, far from being restricted to humans manufacturing experiments, dedifferentiation might be naturally particularly efficient in the context of cancers. Indeed, the debate about the origin of CSCs led to the demonstration that at least some of the CSC might come from non-stem cells (Passegue et al. 2003; Huntly et al. 2004; Krivstov et al. 2006). This means that non-stem cells can become stem cells. Other studies have also highlighted the possibility that stemness would be an outcome of the cancer process (Rapp et al. 2008; Thirant et al. 2011).

None of these crises definitely shut down the established stem cell technoscientific object. Weissmanian central dogma of stem cell biology considering stem cells as stable discrete entities at the head of a non-reversible hierarchy can still be saved. But combined, they really put emphasis on the importance of the question “what kind of property is stemness?” and on the lack of solid answer to it.

Some biologists suggested changes in perspectives on that question. Thus, Loeffler and Roeder (2002) suggested a revision of the classical definition in which they advocated a shift from a “cellular view” to a “system view”. Their aim is “emphasizing stemness as a capability rather than as a cellular property” (p. 13). Considering stemness

as a capability or a disposition lead to take into consideration the environment, which has a major role in the expression or non-expression of the capability. This view has been shared by other biologists (French biologists of the USCI personify this position), arguing that the role of the microenvironment must be taken into account in our view of stem cells, because of the impact it has on the action of stem cells (quiescence, asymmetric division, self-renewal symmetric division or differentiation symmetric division). Stemness, defined as self-renewal and differentiation, “is not really a property that a cell has independent of its environment” argued Lander (2009, p. 70.2) taking the example of glucose that can be converted in very distinct substances, depending on enzymes, temperature, pH, etc... This led him to the conclusion that stemness might even rather be considered as system-level property:

Stemness is a property of systems, rather than cells, with the relevant system being, at minimum, a cell lineage, and more likely a lineage plus an environment. A system with stemness is typically one that can achieve a controlled size, maintain itself homeostatically, and regenerate when necessary (Lander 2009, p. 70.5).

A similar but not identical view holds that stemness must refer to a (set of) function(s) not to an entity. This view is tight with a very original and interesting thesis that stemness “can be induced in many distinct types of cells, even differentiated cells” (Blau et al 2001: 829; see also Zipori 2004, 2005, 2006, 2009, who is a major advocate of this view).

These new perspectives on stemness have been reframed as the “cell state” hypothesis in opposition to an “entity” theory (which is the name given to the orthodoxy) (Fagan Forthcoming; Leychkis et al. 2009). We argue that there are rather four (and probably more) ontologically distinct views on stemness.

- (1) The orthodoxy (let’s call it “entity ontology”) consists in considering stemness as the biological fundamental property of the cells of particular cell types (the stem cells).
- (2) The “capability ontology” can be seen as a revision of the entity theory. It can still support the idea that stemness is a distinctive property of stem cells. But the property itself is view as a “disposition” or “capability”, which might not be

expressed unless the right conditions are met. The consequence is an integration of the microenvironment in the consideration of the property itself. One cannot study stemness without studying the extracellular factors involved in its expression and regulation.

- (3) The “system ontology” abandons the very idea of stem cells and just retains the stemness, which is ascribed to a “system” rather than to a cell, that is a lineage plus its environment.
- (4) The “state ontology” considers the stemness as a cell property, but as the property of any cell (or almost any cell). This might also be called “relational ontology” as has been suggested by Robert (2004, p. 1007) because it is the relation that a cell has with other cells or with a microenvironment that determines its stem or non-stem state.

All these propositions of reevaluation of our ontological understanding of “stem cell” share the idea that the concept of “stem cell” does not refer to an entity like “neuron” or “erythrocyte” does. Question remains to determine if it refers to a capability, a cell or a system?

## **Cutting heads might be endless**

We would like to highlight the practical consequences of those distinct ontological perspectives on stemness.

First let's remember that the coming into being and stabilization of cancer stem cells preceded the appearance of those debates. Thus, the cancer stem cell theory, and more precisely, the model of anti-CSC therapeutic strategy, is based on the entity ontology of stemness. Indeed, the idea that the destruction of the CSCs might led to the definitive elimination of a cancer rely on two presuppositions: first, CSC are somehow qualitatively distinct and distinguishable from other cancer cells; second, stemness is a stable property and differentiation is a one-way street.

What might be the consequences of any change in ontological perspective on stemness?

*What if stemness was a disposition/capability?*

If stemness is a capability, then CSCs can be distinguished from non-CSCs and specifically targeted in order to cure cancer. If succeeded, the outcome would predictably be the same than if stem cells were classical entities: without CSC, cancers that respond to the CSC model won't be able to maintain themselves and will regress and disappear. This would ultimately lead to an effective cure.

This ontological perspective on stemness, however, put emphasis on the comportment of stem cells. This have a particular consequence for elimination of the CSC: any CSCs targeting therapy that would focus on the stemness properties of CSCs might encounter the reductionist problem we have highlighted about the seeking for a genetic portrait of stemness. Indeed, the targeting of CSCs through a functional signature could miss the quiescent CSCs. As far as genetic signatures are concerned, there might be two populations of CSCs rather than one: a population of active CSCs and a population of quiescent CSCs, each presenting a deferent signature. This heterogeneity should be taken into account in the design of new drugs. Only the elimination of all the CSC could achieve the goal of an effective and definitive cure.

The dispositional view also suggests another plausible therapeutic strategy. Indeed, if stemness is a disposition, then cancer stem cells activities rely on the conditions of expression of stemness. Thus, destroying these conditions should lead to the maintenance of the CSC out of the stem state. This should, in turn, lead to the same result than the elimination of the CSC, i.e. disappearance of the cancer cells population, which would no longer be fed by the CSC.

*What if stemness was a system property?*

If stemness is a system property, then cell targeting appears misled since there are no cells to be targeted. This ontological perspective, however, has to be clarified. At current state, the factors involved in the expression of stemness by a system are not clear. What does it mean to be a system property and what would it take to prevent a system like a cancer to express stemness? Furthermore, the difference between the system ontology and the state ontology has to be strengthened. Indeed, one possible interpretation of the system ontology would be that stemness is expressed by some cells but under the control



of the whole system. Let's take a metaphor in order to give an example: in a soccer team, a player may be replaced by another without affecting the tactical scheme. For example, since her retirement the ex-international French feminine player Stéphanie Mugneret-Béghé has been replaced like-for-like by Gaëtane Thiney. The midfielder position and function has been preserved despite the loss of the former player. This metaphor illustrates that in a given system, a function can be attributed to a substitutable item. In this case the loss of the item doesn't affect the system-level function as long as new items can replace the former one. This means that CSC targeting therapies cannot be effective and will lead to relapses through induction of stemness among cancer cells. By contrast, the therapeutic strategy of eliminating the conditions of expression of stemness previously suggested could prove successful.

*What if stemness was a relational property?*

If stemness is a relational property then targeting CSCs might be endless as cancerous non-stem cells could acquire stemness at anytime if the right relation conditions are met. In this case, a CSCs targeting therapies would either never end or likely lead to relapse.

This relational ontology is akin to the second interpretation of the system ontology. Nevertheless, we would like to extend the soccer metaphor to highlight a difference. The relational ontology focuses on an item plus its relations whereas the system ontology focuses on the functions at the system level. In soccer, a player substitution is a good example of the system ontology; a player who endorses a new field position would be an example of the relational ontology. The relational ontology highlights the possibility for an item to acquire a new function. For example, last year, in her new team (ES 16), Stéphanie Mugneret-Béghé has played sweeper or defensive midfielder, depending on several types of "relations" imposing her one or the other of these functions (coaches, team needs, other players' availability and conditions, etc.). The change in focus from the system to the item is far from irrelevant. For example Stéphanie Mugneret-Béghé could probably play every position, but she would be far better as midfielder than as goalkeeper for at least historical reasons: she never trained as a goalkeeper! Biographies of the cells might also have consequences in their ability to express stemness. For example, a red blood cell could never revert to a stem state because they don't have



nucleus anymore. It seems highly probable that cells are unequal in their ability to be dedifferentiated, depending on their morphology, chromatin state and other criteria. From this it follows that a therapeutic strategy based on induction of differentiation might be successful if the differentiation were adequate, at least in certain types of cancers.

## Conclusion

In this chapter, we have tried to show that ontologies of technoscientific objects can be history-laden. The coming into being of cancer stem cell is an example of technoscientific object whose ontology is tightly committed to its selective biographies. We claim that those biographies-laden ontologies can have critical practical consequences. The case of cancer stem cells is paradigmatic. Through their biographical narratives, we have highlighted the “entity” ontology of the CSC. A broader view on stemness shows that the “entity” ontology is in fact competed by other ontological views, for whom supports are recently growing. We have emphasized that these other ontologies cast important doubts on the feasibility and usefulness of the CSC targeting strategy supported by the classical ontology, that raise so many hopes, funding and efforts. They also highlight other plausible therapeutic strategies that might also deserve researches, attentions and funding.

## References

- Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ and Clarke MF (2003) Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 100(7): 3983-3988.
- Arechaga J (1993) On the boundary between development and neoplasia. An interview with professor G. Barry Pierce. *Int. J. Dev. Biol.* 37: 5-16.
- Askanazy M (1907) Die Teratome nach ihrem Bau, ihrem Verlauf, ihrer Genese und im Vergleich zum experimentellen Teratoid. *Verhandl. Deutsch. Pathol.* 11: 39- 82.
- Astaldi G, Allegri A and Mauri C (1947) Experimental investigations of the proliferative activity of erythroblasts in their different stages of maturation. *Experientia* 3(12): 499-500.
- Astaldi G and Mauri C (1950) New criteria to estimate mitotic activity in bone marrow cells.

*Acta Haematologica* 3(2): 122-122.

- Barroca V, Lassalle B, Coureuil M, Louis JP, Le Page F, Testart J, Allemand I, Riou L and Fouchet P (2009) Mouse differentiating spermatogonia can generate germinal stem cells in vivo. *Nat Cell Biol* 11(2):190-196.
- Becker AJ, McCulloch EA and Till JE (1963) Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature* 197: 452-454.
- Belizário JE (2009) Immunodeficient mouse models: An overview” *The Open Immunology Journal* 2: 79-85.
- Blau HM, Brazelton TR and Weimann JM (2001) The evolving concept of a stem cell: entity or function? *Cell* 105(7):829-841.
- Bonnet D and Dick JE (1997) Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 3(7): 730- 737.
- Brawley C and Matunis E (2004) Regeneration of male germline stem cells by spermatogonial dedifferentiation in vivo. *Science* 304(5675): 1331-1334.
- Budde M (1926). Über die Genese der Fetalinkclusionen. *Eitr. Path. Anat.* 75: 357-398.
- Buick RN, Minden MD and McCulloch EA (1979) Self-renewal in culture of proliferative blast progenitor cells in acute myeloblastic leukemia. *Blood* 54(1): 95- 104.
- Burns CE and Zon LI (2002) Portrait of a stem cell. *Dev Cell* 3(5): 612-613.
- Cambrosio A and Keating P (1992a) A matter of FACS: Constituting novel entities in immunology. *Medical Anthropology Quarterly, New series*, 6(4): 362-384
- Cambrosio A and Keating P (1992b) Between fact and technique: The beginnings of hybridoma technology. *Journal of the History of Biology* 25(2): 175- 230.
- Chiba T, Zheng YW, Kita K, Yokosuka O, Saisho H, Onodera M, Miyoshi H, Nakano M, Zen Y, Nakanuma Y, Nakauchi H, Iwama A and Taniguchi H (2007) Enhanced self- renewal capability in hepatic stem/progenitor cells drives cancer initiation. *Gastroenterology* 133(3): 937-950.
- Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, Visvader J, Weissman IL and Wahl GM (2006) Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. *Cancer Res* 66(19):9339-9344.
- Clarkson BD (1969) Review of recent studies of cellular proliferation in acute leukemia. *Natl Cancer Inst Monogr* 30: 81-120.
- Clarkson B, Fried J, Strife A, Sakai Y, Ota K, Okita T (1970) Studies of cellular proliferation in human leukemia. 3. Behavior of leukemic cells in three adults with acute leukemia given continuous infusions of 3H-thymidine for 8 or 10 days. *Cancer* 25(6): 1237-1260.
- Cohnheim J (1875). Congenitales, quergestreiftes Muskelsarkom der Nieren. *Virchows Archiv* 65(1): 64-69.

- Cohnheim, Julius (1889) *General Pathology. A handbook for practitioners and students*. Translation of the second German edition by McKee, Alexander. Section II "The pathology of nutrition". London: The New Sydenham Society.
- Collins AT, Berry PA, Hyde C, Stower MJ and Maitland NJ (2005) Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 65(23): 10946- 10951.
- Colvin GA, Quesenberry PJ and Dooner MS (2006) The stem cell continuum: a new model of stem cell regulation. *Handb Exp Pharmacol* 174: 169-183.
- Cozzio A, Passegue E, Ayton PM, Karsunky H, Cleary ML and Weissman IL (2003) Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors. *Genes Dev* 17(24): 3029-3035.
- Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, Hoey T, Gurney A, Huang EH, Simeone DM, Shelton AA, Parmiani G, Castelli C and Clarke MF (2007) Phenotypic characterization of human colorectal cancer stem cells. *Proc Natl Acad Sci USA* 104(24): 10158-10163.
- Damjanov I and Solter D (1974) Experimental teratoma. *Curr Top Pathol* 59: 69-130.
- Dick JE (1996) Normal and leukemic human stem cells assayed in SCID mice. *Seminars in Immunology* 8(4): 200.
- Duchesneau F (1987). *Genèse de la théorie cellulaire*. Paris, Vrin.
- Dunn GR & Stevens LC (1970) Determination of sex of teratomas derived from early mouse embryos. *J. Nat. Cancer Inst* 44: 99-105.
- Durante F (1874) Nesso fisio-pathologico tra la struttura dei nei materni e la genesi di alcuni tumori maligni. *Arch Memori ed Osservazioni di Chirurgia Practica* 11: 217–26.
- Eramo A, Lotti F, Sette G, Piloizzi E, Biffoni M, Di Virgilio A, Conticello C, Ruco L, Peschle C and De Maria R (2008) Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ* 15(3): 504-514.
- Fagan MB (2007) The search for the hematopoietic stem cell: social interaction and epistemic success in immunology. *Stud Hist Philos Biol Biomed Sci* 38(1): 217-237.
- Fagan, M. (forthcoming) *Philosophy of Stem Cell Biology*. London: Palgrave- Macmillan.
- Fortunel NO, Otu HH, Ng HH, Chen J, Mu X, Chevassut T, Li X, Joseph M, Bailey C, Hatzfeld JA, Hatzfeld A, Usta F, Vega VB, Long PM, Libermann TA and Lim B (2003) Comment on "“Stemness”: transcriptional profiling of embryonic and adult stem cells" and "a stem cell molecular signature". *Science* 302(5644): 393.
- Gavosto F, Pileri A, Gabutti V and Masera P (1967a) Non-self-maintaining kinetics of proliferating blasts in human acute leukaemia. *Nature* 216(5111): 188-189.
- Gavosto F, Pileri A, Gabutti V and Masera P (1967b) Cell population kinetics in human acute leukaemia. *Eur J Cancer* 3(4): 301-307.

- Griffin JD and Löwenberg B (1986) Clonogenic Cells in Acute Myeloblastic Leukemia. *Blood* 68(6): 1185-95.
- Hackney JA, Charbord P, Brunk BP, Stoeckert CJ, Lemischka IR and Moore KA (2002) A molecular profile of a hematopoietic stem cell niche. *Proc Natl Acad Sci USA* 99(20): 13061-13066.
- Hannover, Adolph (1852). *Das Epithelioma, eine eigenthuemliche Geschwulst, die man im allgemeinen bisher als Krebs angesehen hat*. Leipzig.
- Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, Bruns CJ and Heeschen C (2007) Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell* 1(3): 313- 323.
- Huntly BJ, Shigematsu H, Deguchi K, Lee BH, Mizuno S, Duclos N, Rowan R, Amaral S, Curley D, Williams IR, Akashi K and Gilliland DG (2004) MOZ-TIF2, but not BCR-ABL, confers properties of leukemic stem cells to committed murine hematopoietic progenitors. *Cancer Cell* 6(6): 587-596.
- Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA and Lemischka IR (2002) A stem cell molecular signature. *Science* 298(5593): 601-604.
- Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, et al. 2003. Response to Comments on “‘Stemness’: Transcriptional profiling of embryonic and adult stem cells” and “A stem cell molecular signature”. *Science* 302(5644): 393d.
- Kai T and Spradling A (2004) Differentiating germ cells can revert into functional stem cells in *Drosophila melanogaster* ovaries. *Nature* 428(6982): 564- 569.
- Keating P and Cambrosio A (1994) ‘Ours is an Engineering Approach’: Flow Cytometry and the Constitution of Human T-Cell Subsets. *Journal of the History of Biology* 27: 449-479.
- Killmann SA, Cronkite EP, Robertson JS, Fliedner TM and Bond VP (1963) Estimation of phases of the life cycle of leukemic cells from labeling in human beings in vivo with tritiated thymidine. *Lab Invest* 12: 671-684.
- Klebs E (1867) Bemerkungen ueber Larynx-Geschwuelste. *Arch. path. Anat. Phys. klin. Med.* 38: 202-220.
- Kleinsmith LJ and Pierce GB, Jr. (1964) Multipotentiality of single embryonal carcinoma cells. *Cancer Res* 24: 1544-1551.
- Kolata G (2009) Advances Elusive in the Drive to Cure Cancer. *New York Times*, April 23.
- Kraft A (2009) Manhattan transfer: lethal radiation, bone marrow transplantation, and the birth of stem cell biology, ca. 1942-1961. *Hist Stud Nat Sci* 39(2): 171-218.
- Krivtsov AV, Twomey D, Feng Z, Stubbs MC, Wang Y, Faber J, Levine JE, Wang J, Hahn WC, Gilliland DG, Golub TR and Armstrong SA (2006) Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. *Nature* 442(7104): 818-822.

- Lander AD (2009) The 'stem cell' concept: is it holding us back? *Journal of Biology* 8(8):70.
- Langhans T (1867) Ueber Krebs und Cancroid der Lunge, nebst einem Anhang ueber Corpora amylacea in der Lunge. *Arch. path. Anat. Phys. klin. Med.* 38: 497-543.
- Larochelle A, Vormoor J, Lapidot T, Sher G, Furukawa T, Li Q, Shultz L, Olivieri NF, Stamatoyannopoulos G, Dick JE (1995) Engraftment of immune-deficient mice with primitive hematopoietic cells from  $\beta$ -thalassemia and sickle cell anemia patients: implications for evaluating human gene therapy protocols. *Human Molecular Genetics* 4(2): 163-172.
- Larochelle A, Vormoor J, Hanenberg H, Wang JC, Bhatia, M, Lapidot T, Moritz T, Murdoch B, Xiao XL, Kato I, Williams DA, Dick JE (1996) Identification of primitive human hematopoietic cells capable of repopulating NOD/SCID mouse bone marrow: implications for gene therapy. *Nat Med* 2(12): 1329.
- Lawson DA and Witte ON (2007) Stem cells in prostate cancer initiation and progression. *J Clin Invest* 117(8): 2044-2050.
- Lebert, Hermann (1851) *Traité pratique des maladies cancéreuses et des affections curables confondues avec le cancer*. Paris.
- Lenz HJ (2008) Colon cancer stem cells: a new target in the war against cancer. *Gastrointest Cancer Res* 2(4): 203-204.
- Leychik Y, Munzer SR, Richardson JL (2009) What is stemness? *Studies in History and Philosophy of Biological and Biomedical Sciences* 40: 312-320.
- Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF and Simeone DM (2007) Identification of pancreatic cancer stem cells. *Cancer Res* 67(3):1030-1037.
- Lo DC, Allen F and Brockes JP (1993) Reversal of muscle differentiation during urodele limb regeneration. *Proc Natl Acad Sci USA* 90(15): 7230-7234.
- Loeffler M and Roeder I (2002) Tissue stem cells: definition, plasticity, heterogeneity, self-organization and models--a conceptual approach. *Cells Tissues Organs* 171(1):8-26.
- Lorenz E, Uphoff D, Reid TR and Shelton E (1951) Modification of irradiation injury in mice and guinea pigs by bone marrow injections. *J Natl Cancer Inst* 12(1): 197-201.
- Maehle AH (2011) Ambiguous cells: the emergence of the stem cell concept in the nineteenth and twentieth centuries. *Notes Rec R Soc Lond* 65(4): 359-378.
- Maienschein J (2002) Stem cell research: a target article collection: Part II-- what's in a name: embryos, clones, and stem cells. *Am J Bioeth* 2(1):12-19.
- Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Briskin C, Yang J and Weinberg RA (2008) The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 133(4): 704-715.
- Mauer AM and Fisher V (1966) Characteristics of cell proliferation in four patients with untreated

- acute leukemia. *Blood* 28(3): 428-445.
- McCulloch EA (1983) Stem cells in normal and leukemic hemopoiesis (Henry Stratton Lecture). *Blood* 62: 1-13.
- McCulloch EA and Till JE (1981) Blast cells in acute myeloblastic leukemia: a model. *Blood Cells* 7(1): 63-77
- Metcalf D, Moore MA, Warner NL. (1969) Colony formation in vitro myelomonocytic leukemic cells. *J Natl Cancer Inst* 43: 983-1001.
- Mikkers H and Frisen J (2005) Deconstructing stemness. *EMBO J* 24(15):2715- 2719.
- Morel AP, Lievre M, Thomas C, Hinkal G, Ansieau S and Puisieux A (2008) Generation of breast cancer stem cells through epithelial-mesenchymal transition. *PLoS One* 3(8): e2888.
- O'Brien CA, Pollett A, Gallinger S and Dick JE (2007) A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 445(7123): 106-110.
- Passegue E, Jamieson CH, Ailles LE and Weissman IL (2003) Normal and leukemic hematopoiesis: are leukemias a stem cell disorder or a reacquisition of stem cell characteristics? *Proc Natl Acad Sci USA* 100 (Suppl 1) :11842-11849.
- Pierce GB and Dixon FJ Jr. (1959) Testicular Teratomas. I. Demonstration of Teratogenesis by Metamorphosis of Multipotential Cells. *Cancer* 12(3): 573-83.
- Pierce GB (1967) Teratocarcinoma: model for a developmental concept of cancer. *Curr Top Dev Biol* 2: 223-246.
- Pierce GB (1974) Neoplasms, differentiations and mutations. *Am J Pathol* 77(1): 103-18.
- Pierce, G. B. (1977) Relationship between differentiation and carcinogenesis. *J Toxicol Environ Health* 2(6): 1335-42.
- Pierce GB, Nakane PK, Martinez-Hernandez A and Ward JM (1977) Ultrastructural comparison of differentiation of stem cells of murine adenocarcinomas of colon and breast with their normal counterparts. *J Natl Cancer Inst* 58(5): 1329-1345.
- Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, Weissman IL, Clarke MF and Ailles LE (2007) Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Natl Acad Sci USA* 104(3): 973-978.
- Quesenberry PJ (2006) The continuum model of marrow stem cell regulation. *Curr Opin Hematol* 13(4): 216-221.
- Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC and Melton DA (2002) "Stemness": transcriptional profiling of embryonic and adult stem cells. *Science* 298(5593): 597-600.
- Rapp UR, Ceteci F and Schreck R (2008) Oncogene-induced plasticity and cancer stem cells. *Cell Cycle* 7(1): 45-51.
- Remak, R. (1854) Ein Beitrag zur Entwicklungsgeschichte der krebshaften Geschwuelste. *Deutsche*

*Klinik*, 170-174.

Remak, R (1855) *Untersuchungen über die Entwicklung der Wilbelthiere*. R. Reimer, Berlin.

Reya T, Morrison SJ, Clarke MF and Weissman IL (2001) Stem cells, cancer, and cancer stem cells. *Nature* 414(6859):105-111.

Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C and De Maria R (2007) Identification and expansion of human colon-cancer-initiating cells. *Nature* 445(7123): 111-115.

Robert JS (2004) Model systems in stem cell biology. *Bioessays* 26(9): 1005- 1012.

Schleiden MJ (1838) Beiträge zur Phytogenesis. *Archiv für Anatomie, Physiologie und wissenschaftliche Medicin*, 137-176.

Schwann, T. (1839). *Mikroskopische Untersuchungen über die Übereinstimmung in der Struktur und dem Wachstum der Thiere und Pflanzen*. Berlin, Sander'schen Buchhandlung.

Seaberg RM and van der Kooy D (2003) Stem and progenitor cells: the premature desertion of rigorous definitions. *Trends Neurosci* 26(3):125-131.

Shostak S (2006) (Re)defining stem cells. *Bioessays* 28(3): 301-308.

Shults LD, Fumiko I, Greiner DL (2007) Humanized mice in translational biomedical research. *Nat Rev Immunol* 7: 118-30.

Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J and Dirks PB (2003) Identification of a cancer stem cell in human brain tumors. *Cancer Res* 63(18):5821-5828

Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD and Dirks PB (2004) Identification of human brain tumour initiating cells. *Nature* 432(7015):396-401.

Stevens LC (1968) The development of teratomas from intratesticular grafts of tubal mouse eggs. *J. Embryol. Exp. Morph* 20: 329-341.

Stevens LC (1970) The development of transplantable teratocarcinomas from intratesticular grafts of pre- and postimplantation mouse embryos. *Dev Biol* 21(3): 364-382.

Suetsugu A, Nagaki M, Aoki H, Motohashi T, Kunisada T and Moriwaki H (2006) Characterization of CD133+ hepatocellular carcinoma cells as cancer stem/progenitor cells. *Biochem Biophys Res Commun* 351(4): 820-824.

Szotek PP, Pieretti-Vanmarcke R, Masiakos PT, Dinulescu DM, Connolly D, Foster R, Dombkowski D, Preffer F, Maclaughlin DT and Donahoe PK (2006) Ovarian cancer side population defines cells with stem cell-like characteristics and Mullerian Inhibiting Substance responsiveness. *Proc Natl Acad Sci USA* 103(30): 11154- 11159.

Thirant C, Bessette B, Varlet P, Puget S, Cadusseau J, Tavares Sdos R, Studler JM, Silvestre DC, Susini A, Villa C, Miquel C, Bogeas A, Surena AL, Dias-Morais A, Leonard N, Pflumio F, Bieche I, Boussin FD, Sainte-Rose C, Grill J, Daumas-Duport C, Chneiweiss H and Junier MP (2011) Clinical relevance of tumor cells with stem-like properties in pediatric brain tumors. *PLoS One*



6(1): e16375.

Till JE and McCulloch EA (1961) A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res* 14: 213-222.

Till JE and McCulloch EA (1980) Hemopoietic stem cell differentiation. *Biochim Biophys Acta* 605(4): 431-459.

Virchow R (1855) Über Perlgeschwuelste (Cholesteatoma Joh. Mueller's). *Arch path Anat Phys klin Med* 8: 415.

Virchow R. (1858) *Die Cellularpathologie in ihrer Begründung auf physiologische und pathologische Gewebelehre*. Berlin : August Hirschwald.

Vogel G (2003) Stem cells. 'Stemness' genes still elusive. *Science* 302(5644): 371.

Weissman IL, Heimfeld S and Spangrude G (1989) Haemopoietic stem cell purification. *Immunol Today* 10(6): 184-185.

Willis, RA (1948) *Pathology of Tumours*. London : Butterworth & Co.

Younes, S. A. et al. (2003) HIV-1 viremia prevents the establishment of interleukin 2-producing HIV-specific memory CD4+ T cells endowed with proliferative capacity. *J. Exp. Med.* 198, 1909–1922.

Zipori D (2004) The nature of stem cells: state rather than entity. *Nat Rev Genet* 5(11): 873-878.

Zipori D (2005) The stem state: plasticity is essential, whereas self-renewal and hierarchy are optional. *Stem Cells* 23(6): 719-726.

Zipori D (2006) The stem state: mesenchymal plasticity as a paradigm. *Curr Stem Cell Res Ther* 1(1): 95-102.

Zipori D (2009) *Biology of stem cells and the molecular basis of the stem state*. Dordrecht; New York: Humana Press.